Evaluation of Anticonvulsant Activity of Novel Synthetic 1,2,4-Triazole Containing Benzoxazole Derivative.

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Abstract

Background:

There are various pharmacological activities associated with benzoxazole, and alterations in the nucleus of benzoxazole have resulted in a number of compounds that have differing pharmacological properties. Triazole may be added to it to explore potential central nervous system (CNS) activities. There is potential for an antiepileptic effect with the triazole substituted benzoxazole derivative (TSBD) tested in this study. Biologically active compounds are prepared by focusing on the construction of molecules that are biologically active.

Methods: The animals (Swiss albino mice) of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water ad libitum, after obtaining ethical committee approval. The present study was aimed to evaluate the anticonvulsant activity of TSBD derivative in mice. Anticonvulsant

activity of TSBD was evaluated against maximal electroshock (MES) and pentylenetetrazole (PTZ)-induced induced convulsions in mice.

Results: In these studies, TSBD at 50,100 and 200 mg/kg showed significant and dose-dependent inhibition of MES and PTZ -induced convulsions.

Conclusion: In conclusion, TSBD showed potent anticonvulsant activity, and possible mechanism may be due to enhanced GABA levels in the brain.

Keywords: Epilepsy, Maximal electroshock, Pentylenetetrazole, Benzoxazole, Triazole

Introduction

Epilepsy is one of the most prevalent neurological disorders affecting approximately 1% of people across the globe [1]. Epilepsy risk is 3.9% over a lifetime, with males having a slightly higher risk [2]. Epilepsy affects less than 1% of the U.S. population at any given time, with a disproportionate impact on infants and elderly people due to the fact that many people (especially children) become seizure free [3]. According to estimates, epilepsy costs the healthcare system $15.5 billion annually [4]. The economic burden is further aggravated when patients with epilepsy miss work or school as a result of their illness [5]. Generally, seizures are classified as either generalized (tonic-clonic, involving both hemispheres and multiple structures) or focal. It is possible for focal seizures to spread widely or to be localized in one hemisphere exclusively [6]. Several antiepileptic drugs are available for the treatment of epilepsy [7].
Pregabalin, tiagabine, stiripentol, levetiracetam, zonisamide, lamotrigine, and topiramate are just a few new antiepileptic medications that are severely compromised by severe side effects like vertigo, ataxia, headache, hirsutism, hepatotoxicity, gastrointestinal problems, cardiovascular issues, and, particularly, uncontrolled seizures, which affect about 30% of patients [8].

It is unclear what causes human epilepsy at the cellular level or how most antiepileptic medications work because of their complicated modes of action. The complex modes of action of the majority antiepileptic medicines and the incomplete understanding of the cellular mechanisms underlying human epilepsy make it challenging to apply rational techniques in the field of drug discovery. New, less hazardous, and more potent antiepileptic medications are

desperately needed [9].

Due to their numerous pharmacological actions, benzoxazole derivatives have taken a special place in medicinal chemistry. Benzoxazole was found to be a fundamental component for more intricate and bioactive compounds. The benzoxazole nucleus has been modified to produce a wide range of compounds with various pharmacological properties. The benzoxazole moiety is investigated and explored for its pharmacological significance in diverse situations. Numerous benzoxazole derivatives have been discovered and are known to have powerful antibacterial and antifungal properties [10], as topoisomerase-I inhibitors [11], HIV-1 reverse transcriptase inhibitors [11], anticancer properties [12], and as treatments for Alzheimer's disease [13].

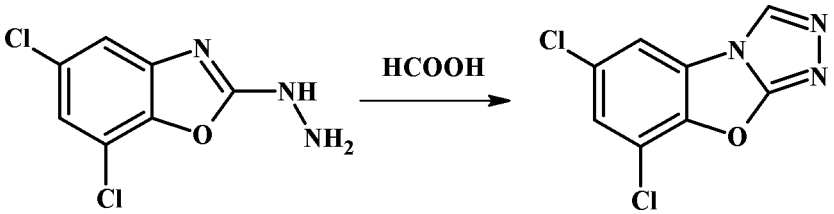
Given the significance of the benzoxazole moiety in pharmacology, it is important to examine the biological activities of the derivatives in various animal models to see whether they have any potential for use in medicine. In order to better understand the potential effects of the substituted compounds on the central nervous system (CNS), a triazole nucleus with a planer five-membered heterocyclic system and three nitrogen atoms—one pyrrole and two pyridine type—can be added to the benzoxazole moiety [14]. 1,2,3 and 1,2,4 are the two structurally isomeric forms of triazole [15]. One of these two compounds, 1,2,4-triazole, has been researched for a variety of pharmacological activities. Alprazolam (a sedative) [16], etoperidone (an antidepressant) [17], and nefazodone (an antidepressant, a 5-HT2 antagonist) [18] are 1,2,4-triazole compounds that have found use in medicine. Since serotonin receptors can alter the ionic conductance and/or concentration within brain cells to directly or indirectly depolarize or hyperpolarize neurons. The excitability of the majority of epilepsy-related networks may be altered by 5-HT [19]. Its anticonvulsant properties are based on the actions of nefazodone (5-HT2 antagonist) [18] and other substituted 1,2,4-triazole derivatives [20].

As a result, the test chemical, 6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole, a triazole substituted benzoxazole derivative (TSBD), may have anticonvulsant properties. The current investigation will therefore use Swiss albino mice to investigate the anticonvulsant effectiveness of a novel synthetic benzoxazole derivative in seizures caused by PTZ and maximum electroshock.

Materials and methods:

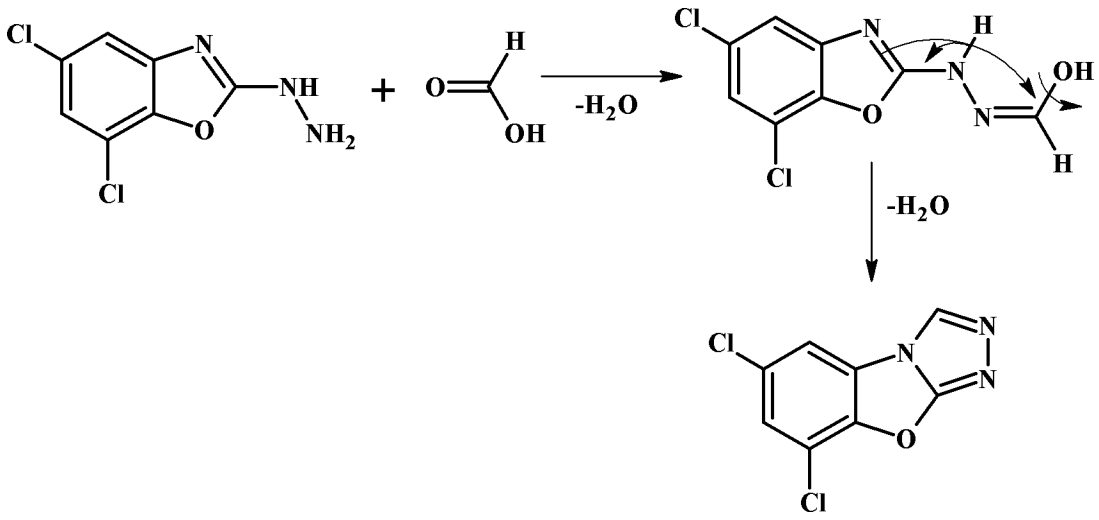
Preparation of 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole

The creation of powerful compounds for application in biology was the emphasis of the research. Numerous pharmacological properties are associated with triazoles. As a result, the intermediate was reacted with formic acid to become triazole. The reaction and its mechanism are mentioned in the scheme-1 and scheme-2 respectively [21].



5,7-dichloro-2-hydrazino-1,3-benzoxazole **6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole**

Scheme-1



Scheme-2

The structure of the product 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole was

confirmed by the spectral studies. It showed the absence of both -NH and -NH_2 groups in its IR spectrum, ^{1}H NMR and mass spectral studies. In the IR spectrum, the stretching frequency at 3367 cm^{-1} and 3481 cm^{-1} disappeared (fig.1). A peak at 4.6 for two protons of -NH_2 and at 9.3 for a proton of -NH disappeared and a new peak was observed at 12.18 confirmed the structure. The signal obtained for proton at 7.17 (s, 1H, Ar H) 7.35 (s, 1H, Ar H) and at 12.18 (s, 1H, for -N=CH) confirmed the formation of compound. Mass spectrum at M^+ (228), M^{+2} (230) and M^{+4} (232) corresponds to its molecular weight.

Animals

The animals (Swiss albino mice) 20–25 g of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water ad libitum. Permission and approval for animal studies were obtained from the K.M.College of pharmacy,Madurai.

Acute toxicity studies

In Albino Wister rats, the TSBD were examined for their acute toxicity profile with relation to behavioral characteristics. Following recommendations from the Organisation for Economic Co-operation and Development (OECD), the limit test doses of 300 and 2000 mg/kg body weight were employed. The acute oral toxicity study carried out in accordance with OECD recommendations demonstrates the TSBD's non-toxic nature. The test animals' regular behavior over a 14-day period implies that the aforementioned unique synthetic benzoxazole is not harmful. Therefore, TSBD may be safe up to a dose of 2000 mg/kg of animal body weight [22].

Anticonvulsant activity

Maximal electroshock (MES) induced seizures

Maximal electroshock seizure model was used to evaluate the anticonvulsant activity of TSBD. Seizures were induced in mice by delivering electroshock of 50mA for 0.2 seconds by means of an electro- convulsion meter through a pair of ear clip electrodes [23]. The test animals were divided into five groups (n=6) the animals of the respective groups were treated with vehicle,

50,100, 200 mg/kg of TSBD through intraperitoneally and standard group received phenytoin (25 mg/kg) injected i.p. [24] and tested after 30 minutes for MES induced seizure response.

Pentylenetetrazole (PTZ) induced seizures

PTZ at the dose of 80 mg/kg (minimal dose needed to induce convulsions) was injected i.p. to induce clonic-tonic convulsions in mice. The test animals were divided into five groups (n=6) the animals of the respective groups were treated with vehicle, 50,100, 200 mg/kg of TSBD through intraperitoneally and standard group received phenytoin (25 mg/kg) injected i.p and PTZ was injected i.p. 60 min after the administration of drug. Occurrence of hind-limb tonic extension (HLTE) and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected [25].

Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using ANOVA (One-way) followed by a post test (New mann keuls multiple range tests). Differences between means were considered significant at 5% level of significance ($P \leq 0.05$).

Results

In the present study, TSBD (50,100 and 200 mg/kg, ip) was evaluated for anticonvulsant activity against MES induced and PTZ convulsions in mice.

In MES-induced seizures, Swiss albino mice pretreated with the TSBD have been significantly protected from convulsions induced by electroshock one-hour post-dosing. The inhibition achieved at the doses 50,100 and 200 mg/kg ($p<0.001$). TSBD dose dependently prolonged the onset of convulsions in the treated group compared to control group (Table 1).

PTZ-induced seizures, animals treated with TSBD at a dose of 50,100 and 200 mg/kg ($p<0.001$) showed alteration in the occurrence of HLTE and duration of seizures significantly as related to controls in the model of convulsion induced by pentylenetetrazole in mice (Table 2).

Discussion

According to data from the current trial, TSBD dramatically shortens the time it takes for electroconvulsive shock seizures to start and reduces how severe they are. The study also demonstrates that PTZ considerably delayed the start of tonic convulsion.

The effect of MES on sodium influx is well-known, as it opens sodium channels and increases glutamate levels, an excitatory amino acid. In humans, glutamate triggers symptoms identical to those of petit mal epilepsy by acting on NMDA receptors [26]. The agents that block voltage-dependent sodium channels (phenytoin, sodium valproate, felbamate, and lamotrigine) and/or those that reduce levels of excitatory amino acids and/or antagonize their actions have all been shown to be effective in MES-induced epilepsy model (e.g., felbamate) [27]. Further, PTZ has been shown to be a potent antagonist of the GABA receptor site, and its administration results in dramatic reductions in GABA levels and GABAA receptor density in various brain regions [28], causing continuous stimulation of neurons in the cerebral cortex and convulsions that mimic human absence seizures exactly [29].

One of the benzoxazole derivative 4- (2- (alkylthio)benzo[d]- oxazol- 6- yl)- 2H- 1,2,4- triazol- 3(4H)- one was tested for antiepileptic activities with MES and PTZ models and the result was positive with MES model. Result suggested that anticonvulsive property of the compound was by GABA content enhancement or may be by regulating the GABA function in brain. Also, docking study result shows the presence of test compound in the BZD-binding site of GABA receptor which confirms that compound has similar binding to diazepam [30].

TSBD having benzoxazole in the structure it may bind to the BZD-binding site on GABA receptor, delayed the occurrence of convulsions and reduction in the seizures duration, it is feasible that it may interfere with gabaergic mechanism to exert their anticonvulsant effect.

All the tested doses of TSBD (50,100 and 200 mg/kg) showed potent anticonvulsant activity in both MES and PTZ models; further, the protective effect of TSBD was found to be significant ($p<0.001$) and dose-dependent.

Conclusion

The TSBD is regarded to be a new treatment approach for treating both grand mal and petit mal epilepsy as a result of these findings, which indicate that it displays considerable anticonvulsant effectiveness against both MES and PTZ -induced convulsion models. In order to better understand the chemical mechanism behind the anticonvulsant effect of TSBD, additional research is being conducted. One of the potential processes is hypothesized to be linked to the modulation of GABA levels in the brain.

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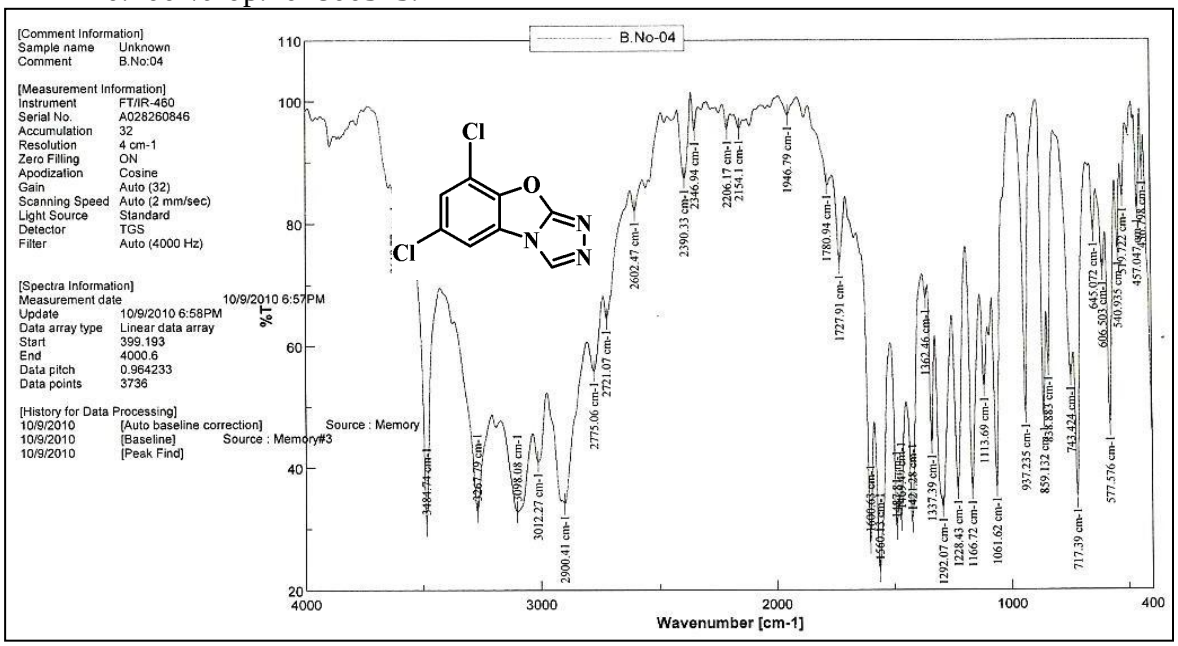

Fig.1 IR spectrum of 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole

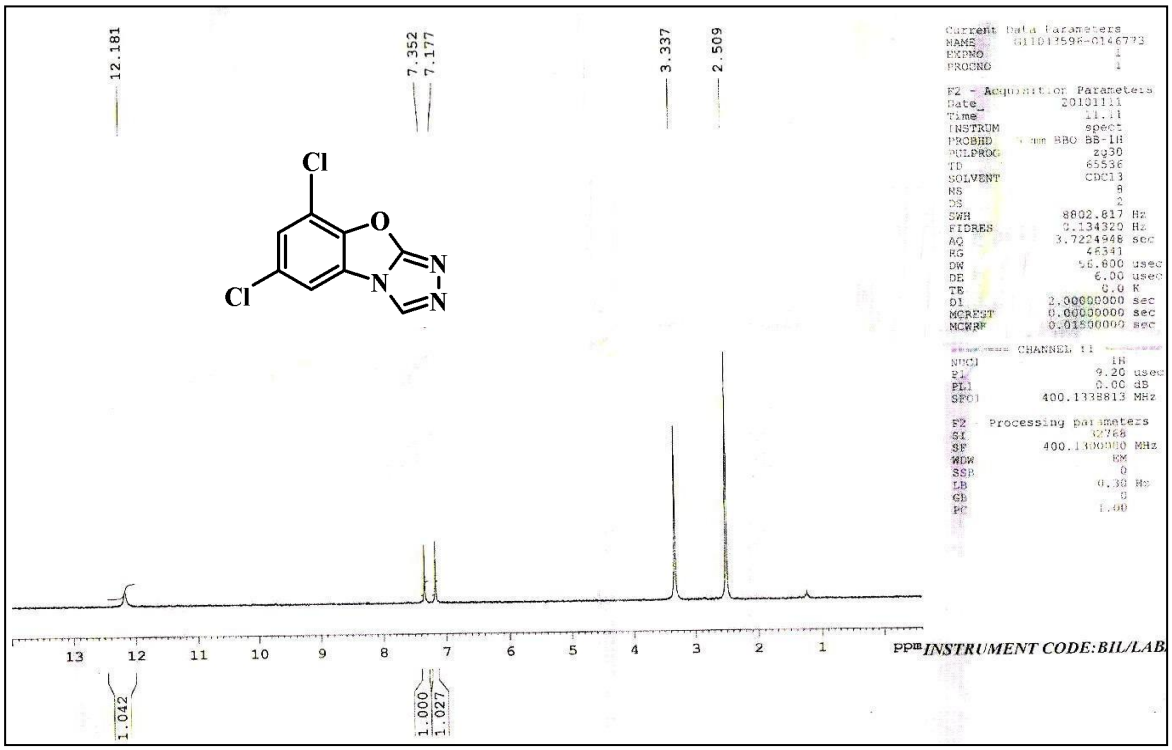

Fig.2 ^{1}H NMR spectrum of 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole

Table 1: Effect of TSBD on Hind limb extension induced by MES in mice.

Groups	**Treatment**	**Hind limb extension (Sec)**
Group-1	Normal saline, 2 ml/kg p.o.	13.8 ± 1.18
Group-2	Phenytoin (25 mg/kg, I.P)	0.24 ± 0.12*a
Group-3	TSBD -50mg/kg.I.P	4.08 ± 0.85*a
Group-4	TSBD -100mg/kg.I.P	3.45 ± 0.67*a
Group-5	TSBD -200mg/kg.I.P	2.60 ± 0.55*a

Note: Percent inhibition expressed as mean ±SEM.
*a- values are significantly different from Normal control
P <.0001, considered as extremely significant

Table 2: Effect of TSBD at different doses on PTZ induced seizures in mice.

Groups	**Treatment**	**Onset Time (Sec)**	**Duration of HLTE (Sec)**
Group-1	Normal saline, 2 ml/kg p.o.	52.65 ± 0.22	38.36 ± 0.53
Group-2	Phenytoin (25 mg/kg, I.P)	00 ± 00*a	00 ± 00*a
Group-3	TSBD -50mg/kg.I.P	58.20 ± 0.48*a	33.20 ± 0.35*a
Group-4	TSBD -100mg/kg.I.P	63.15± 0.56*a	30.57 ± 0.30*a
Group-5	TSBD -200mg/kg.I.P	66.10 ± 0.66*a	26.19 ± 0.23*a

Note: Percent inhibition expressed as mean ±SEM.
*a- values are significantly different from Normal control
P <.0001, considered as extremely significant